

## REPORT DOCUMENTATION PAGE

0146

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1. REPORT DATE (DD-MM-YYYY) 31/03/2005		2. REPORT TYPE Final Technical		3. DATES COVERED (From - To) 01/01/2002 - 31/12/2004	
4. TITLE AND SUBTITLE  Pulsed Electric Fields for Biological Weapons Defense				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER F49620-02-1-0073	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)  Gundersen, Martin A.				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)  University of Southern California Electrical Engineering-Electrophysics 3737 Watt Way, PHE 512 Los Angeles, CA 90089-0271				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) USAF, AFRL Air Force Office of Scientific Research 4015 Wilson Blvd, Room 713 Arlington, VA 22203-1954				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT  Approved for public release: distribution is unlimited.					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The project has demonstrated that ultra-short, high-field pulses are a useful tool for study of cellular responses. The research has established that intracellular effects including apoptosis can be induced by the application of short, intense (but low total energy) electric pulses, and has seen variability in cell response. Experiments on human cells have produced convincing evidence that these applied fields nondestructively alter subcellular processes and can be investigated using biophotonic studies for imaging of morphological and functional changes at subcellular levels. In particular, it is clear that there are a range of responses to intense, ultra-short pulses, and that many lines of spores and cells require study. Technology for the application of pulses has been developed, and results of studies of toxicity have been undertaken, including detailed studies of <i>Bacillus atrophaeus</i> (formerly <i>Bacillus subtilis</i> var. <i>niger</i> ).					
15. SUBJECT TERMS nanosecond high-field electric pulse, electroperturbation, electroporation, pulse-Induced phospholipid translocation, nanoelectropulse					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT  UL	18. NUMBER OF PAGES  11	19a. NAME OF RESPONSIBLE PERSON Dr. Robert Barker
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code) 703-696-8574

20050707 014

## **"Pulsed Electric Fields For Biological Weapons Defense"**

AFOSR Grant No. F49620-02-1-0073

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### **OBJECTIVES**

The objective will be to conduct electroporation experiments using a range of electrical pulse parameters to

- determine the response of cells to the fields
- determine effectiveness of responses to tailored electromagnetic fields
- develop technology for application of pulses to small enclosed containers and envelopes
- determine effect of the application of ultra-short pulses on intracellular gene expression leading to toxicity for spores and bacteria, and on the integrity of cytoplasmic and internal membranes and other intracellular structures

### **STATUS OF EFFORT**

Materials and apparatus have been procured and assembled and a basic experimental plan has been devised for studies of inactivation of bacterial spores in envelopes by exposure to ultrashort, high-field, electric pulses. A rapid (less than 24 hours), qualitative (growth, no-growth) assay based on a commercial sterility monitoring kit, which utilizes spores of *Bacillus atrophaeus* (formerly *Bacillus subtilis* var. *niger*) deposited on paper in glassine envelopes, has been implemented with simple laboratory equipment. A team has been trained to operate the pulse generator and to perform the microbiological assay.

An initial series of experimental exposures has confirmed the applicability of the assay method and provided some preliminary indications of the relative resistance of bacillus spores in envelopes to inactivation by high electric fields after longer exposures. Ongoing work is addressing issues of threshold fields for apoptotic induction. Data obtained in other cells indicates such threshold effects; in studies of Jurkat T cells we have observed data for field strength threshold for phosphatidylserine externalization, and evidence for absence of a pulse rate repetition effect from 2 to 2000 hertz. Real-time visualization of these effects are now underway.

## ACCOMPLISHMENTS/NEW FINDINGS

Described in this report are results of spore studies, and supporting research. This includes primarily research into pulsed power supporting these studies (that is, detail about pulse generators incorporated into a microscope for real-time investigations). Research in detail about nanoelectropulse investigations into cell lines is provided in a companion report for the grant “Ultra Short Pulse Electroporative Physics and Technology”, AFOSR Grant No. F49620-01-1-0495.

We reported at Electromed 2003 and in the 2003 report a study of the application of ultra-short high-field electric pulses (5 MV/m, 100-ns pulse width, 4-ns rise time) to *Bacillus atrophaeus* spores deposited on a paper strip in a sterile glassine envelope to investigate the utilization of pulsed power systems for sterilization of paper and printed material in applications such as mail handling systems. The effectiveness of pulsed fields of high instantaneous power, but low energy, is evaluated as a function of number of pulses for non-thermal, non-chemical spore inactivation in a dry environment at ambient temperature. This system is under consideration as an alternative to gamma irradiation, electron beams, and chemical agents. Germination of bacterial spores incubated after electric field exposure was qualitatively measured in terms of the color (pH) change and the turbidity of the medium every two hours up to twenty-four hours. The reduction in the spore germination rate varied with pulse dosage, but was reduced only up to 50 % at a 16-hour time point. Doses up to ten millions pulses delivered over a period of 1000 seconds delayed the onset of germination or reduced the rate of germination but did not sterilize the spore samples used in these experiments.

Bacteria spores deposited on paper strips were incubated in an indicator medium at 37 °C after electric field exposure. Germination was qualitatively measured in terms of the color change and the turbidity of the medium. When the bacterial spores germinate, they respire and produce CO<sub>2</sub>, which lowers the pH of the medium. As more CO<sub>2</sub> is produced, the color of the indicator changes from orange to yellow. We recorded indicator color change every two hours for 24 hours. When the bacterial spores germinate, they detach from the paper strips and begin to multiply in the indicator medium. As the population of bacteria increases, the medium becomes cloudy. With a fixed initial inoculum and controlled incubation temperature, the turbidity at a given time is a function of the number of spores that germinated. We observed turbidity every two hours for 24 hours). At any given time after pulse exposure, samples exposed to a greater number of pulses showed less growth. For any pulse dose, the reduction in the number of germinating spores, or in the germination time of surviving spores, was ≤50 % at a 16-hour time point.

**Pulse Generation for Advanced Studies:** Applying pulses with high voltage (or field), high power, and fast (turning on in nanoseconds) requires design of advanced pulse generation, transmission, and coupling to the electrical load, typically cells in culture, or tissue, which has been a central part of the research. The principal issues encountered include distortion of nanosecond pulses due to the high frequency characteristics, and the need to retain high field along with high speed. Fast rising pulses require advanced switching elements such as MOSFETs and other pulsed power switches, incorporated into a suitable pulse generation architecture.

Research for field-response biological applications includes MOSFET pulse generator development [Behrend et al. 2002, 2003, 2004], pulse transmission and related switching issues [Gu et. al., 2002, 2003 a,b,c, 2004]; modeling of pulsed power systems for biological applications [Wijetunga et al. 2003], advanced designs for nanoelectropulse pulsers [Kuthi et al. 2003, 2004] catheters [Thu et al. 2004], and non-invasive aspects [Sun et al. 2004]. Research into the development of the pulse generator-types has included:

Pulse generation for real-time microscope observations of cells. This required specialized pulse generation equipment for integration into single cell optical studies (“Minipulser” and “Micropulser”),

Preliminary development of smaller pulse generators for various applications. This included short pulse equipment based on fast recovery diodes, and background and preliminary work modeling pulsed power devices for short pulse generation (Blumlein).

Investigation of frequency response issues for catheters (to determine approaches that will allow pulses to propagate undistorted, and transfer ns rise time to samples).

**Micropulser for Microscope Studies** The MicroPulser (‘MicroChamber’ slide fabricated for sampling cells shown in Figure 1, MicroPulser shown in Figure 2) was developed as a compact solid state pulse generator designed for optical studies of cell electroperturbation [Behrend et al. 2002, 2003, 2004]. Data can be collected from a microscope slide sample (Figure 1) at any time during and after pulsed electric field exposure. The MicroPulser system offers flexibility in pulse parameters and interface to optical instruments that are not possible with cuvette pulse generator systems. The most critical pulse parameters for electroperturbation are fast rise time, amplitude, and width. The micropulser is designed to provide extreme flexibility in these parameters along with maximum 25 MHz repetition rate. The micropulser aims for both miniaturization and flexibility for any pulse width as a single-MOSFET output stage pulse generator.

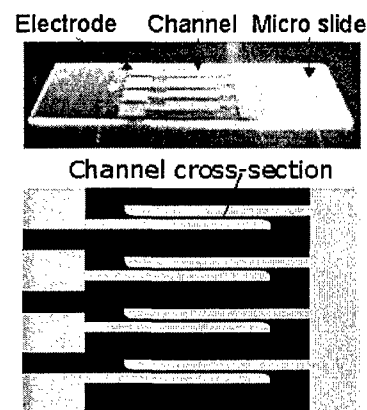


Figure 1. Microchamber fabricated on microscope slide.

The load for the micropulser is a glass slide having deposited gold electrodes that form channels 25  $\mu\text{m}$  wide, 25  $\mu\text{m}$  deep, and 20 mm in length (Figure 1). Cells suspended in liquid growth medium are pipetted into the channels. The growth medium within one such channel presents an electrical load of 37 ohms in parallel with 14 pF. A microscope slide in process of fabrication has two channels 25  $\mu\text{m}$  wide and two channels 50  $\mu\text{m}$ , giving a total parallel load of 12 ohms in parallel with 42 pF. The instrumented

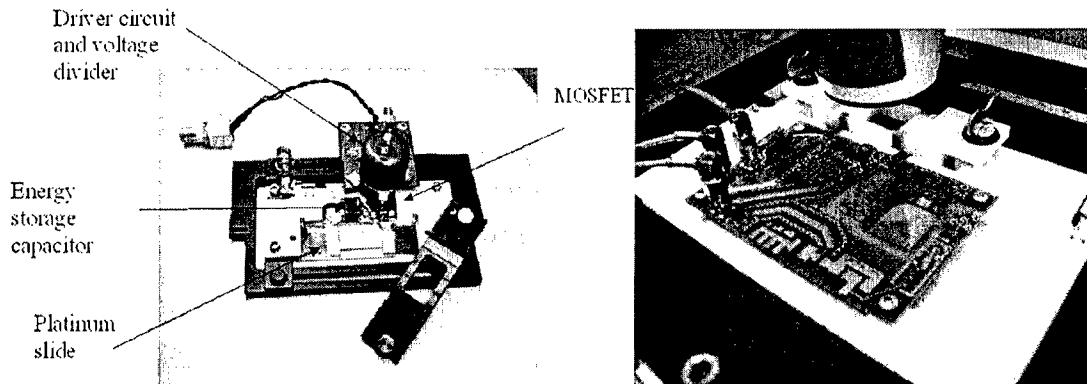


Figure 2: Two versions of micropulsers. Early version of the MicroPulser unit with a platinum foil microchamber slide in position for pulse exposure. Right: Recent version, mounted on stage, in Zeiss Axiovert inverted microscope.

microscope slide is comprised of gold deposited electrode lines separated by 25 - 100  $\mu\text{m}$  and covered by a second glass slide hold the cell solution. The electrical load impedance is 200 - 400  $\Omega$ .

To meet the demand for real-time observation of electroperturbation the microscope slide and micropulser unit must fit on the stage of an inverted fluorescence microscope. The pulse generator should have all RF power devices on stage, leaving only DC power source and trigger signal source for external equipment. Additionally, the fast rise time requirements necessitate short current paths for low inductance. Furthermore, components should be surface mount and coplanar over the ground plane. A MOSFET switched capacitor is well matched to the physical dimensions of the working environment. Mechanically, the MicroPulser is a custom stage insert to the microscope (Fig. 2). The MOSFET, capacitor, and slide contact electrodes all mounted in a compact coplanar arrangement (Fig. 2). Pressure to the mating electrodes of the slide is applied by a windowed cover plate fastened over the slide. The gate drive circuit to the MOSFET and the voltage divider comprise a daughter board mounted over the switching MOSFET. No additional heat sinking is required for the MicroPulser power components since it is an extremely low average power pulse generator and no component heating occurs during the short pulse burst.

**Advanced architectures:** We have conducted preliminary studies of a smaller pulse generation system based on a different, solid state architecture employing fast-recovery diodes and shorted transmission lines (“nanopulser”) [Kuthi et.al., 2004]. This generator produces 3.5 ns wide,  $\pm 350$  V amplitude bipolar pulses into 50-ohm load at the maximum repetition rate of 100 kHz.

**Catheter Preliminary Studies:** A key issue for pulse generation and application is the transmission of pulses without distortion. This is a non-trivial issue for the short pulses required, because many approaches to pulse transmission have bandwidth limitations well below 1 GHz. Therefore, initial design of cathodes required investigation of bandwidth limitation of designs. Studies of catheters [Thu 2004] therefore included investigation of the propagation properties at frequencies appropriate for nanosecond pulses, and included measurements with commercially available RF ablation catheters. Presently, radiofrequency (RF) is used to treat diseases by resistively heating the diseased tissues. RF technology limitations include, for example, rise of tissue temperature to about 100 °C at the electrode tip causing sudden impedance increase, which obstructs further lesion growth. Continuous ablation at 100 °C gives rise to coagulation of blood and charring of the tissues. The input impedances for open and short terminations of various lines (catheters) were measured over a frequency range of 1 MHz to 500 MHz using a network analyzer. The data was processed using open-short method for characteristic impedance calculation. At frequencies above 1 MHz, the catheters show decreasing characteristic impedance. In addition, a square wave of pulse width 10 ns, amplitude of rise time of 200 ps was delivered into the catheter and the output recorded on an oscilloscope to investigate the distortion of ultra-short pulses by the catheter. A square wave of pulse width 10 ns, amplitude of 4 V and rise time of 200 ps is delivered into the catheter and the output recorded on an oscilloscope to investigate the distortion of ultra-short pulses by the catheter. Input and output waveforms processed by Matlab are transformed to the frequency domain to determine the transfer function of the catheter. By inverse Fourier transform, the time-domain transfer function is obtained and convolved in Matlab® with several input pulse shapes of interest to predict the output pulse .

For minimally invasive *in vivo* treatment applications, evaluation of commercially available microwave cables and RF ablation catheters for compatibility with ultra-short pulse cancer therapy demonstrates that RF ablation catheters distort high frequency components of nanosecond pulses. Thus, nanosecond pulses will lose the fast rise time that is necessary to implement the approach.

These results delineate pulse generation circuits for development and integration into studies of biological and spore response to ultra-short intense electric pulses.

## PERSONNEL SUPPORTED

Professor Martin Gundersen  
Dr. Andras Kuthi  
Mr. Tom Vernier  
Mr. Matthew Behrend  
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Mr. Yushun Zhang

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Graduate Research Assistant  
Graduate Research Assistant  
Graduate Research Assistant  
Research Lab Technician

## PUBLICATIONS

Various of these publications and presentations were supported in part by other grants as well, including, principally, the AFOSR support for bio-inspired concepts (AFOSR Grant No. F49620-01-1-0495 described in a separate report).

### *Journal Papers*

Vernier, P. T., A. Li, L. Marcu, C. M. Craft, and M. A. Gundersen, “Ultrashort pulsed electric fields induce membrane phospholipid translocation and caspase activation: differential sensitivities of Jurkat T lymphoblasts and rat glioma C6 cells, *IEEE Trans. Dielect. Elect. Ins.* 10:795–809, 2003.

Vernier, P. T., Y. Sun, L. Marcu, S. Salemi, C. M. Craft, and M. A. Gundersen, Calcium bursts induced by nanosecond electric pulses, *Biochem. Biophys. Res. Commun.* 310:286-295, 2003.

Vernier, P. T., M. Thu, L. Marcu, C. M. Craft, and M. A. Gundersen, Nanosecond electroperturbation -mammalian cell sensitivity and bacterial spore resistance,” *IEEE Trans. Plasma Sci.* 32, pp. 1620-1625, August 2004.

Vernier, P. T., Y. Sun, L. Marcu, C. M. Craft, and M. A. Gundersen, Nanoelectropulse-induced phosphatidylserine translocation, in press, *Biophys. J.* 86, pp. 4040-4048, 2004.

Vernier, P. T., Y. Sun, L. Marcu, C. M. Craft, and M.A. Gundersen, “Nanosecond Pulsed Electric Fields Perturb membrane Phospholipids in T Lymphoblasts,” *FEBS Letters*, 572:103-108, 2004.

Behrend, M. A. Kuthi, X. Gu, P.T. Vernier, L. Marcu, M. Gundersen, “Pulse generators for pulsed electric field exposure of biological cells and tissues,” *IEEE Trans. on Dielectrics and Electrical Insulation*, 10:820-825, 2003

Shamiloglu, E., Barker, R.J., Gundersen, M., and Neuber, A.A., “Modern Pulsed Power: Charlie Martin and Beyond,” *IEEE Proceedings*, Volume 92, Issue 7, July 2004, pp. 1014-1020.

Gaudet, J.A., Barker, R.J., Buchenauer, C.J., Christodoulou, C., Dickens, J., Gundersen, M.A., Joshi, R.P., Krompholz, H.G., Kolb, J.F., Kuthi, A., Larousssi, M., Neuber, A., Nunnally, W., Schamiloglu, E., Schoenbach, K.H., Tyo, J.S., Vidmar, R.J., “Research issues in developing compact pulsed power for high peak power applications on mobile platforms,” *Proceedings of the IEEE*, Vol. 92, Issue 7, July 2004, pp. 1144-1165.

*Papers in Conference Proceedings*

Behrend, M., Kuthi, A., Vernier, P. T., Marcu, L., Craft, C., and Gundersen, M., “Micropulser for Real-Time Microscopy of Cell Electroperturbation,” Proceedings of the 2002 International Power Modulator Conference and High Voltage Workshop, pp. 358-361.

Kuthi, A., Vernier, T., Gu, X., and Gundersen, M., “Compact Nanosecond Pulse Generator for Cell Electroperturbation Experiments,” Proceedings of the 2002 International Power Modulator Conference and High Voltage Workshop, pp. 354-357.

Gu, X., Myles, C. W., Kuthi, A., Shui, Q., and Gundersen, M. A., “Gallium Arsenide and Silicon FET-Type Switches for Repetitive Pulsed Power Applications,” Proceedings of the 2002 International Power Modulator Conference and High Voltage Workshop, pp. 437-440.

P. Wijetunga, X. Gu, A. Kuthi, P. T. Vernier, M. Behrend and M.A. Gundersen, “Electrical Modeling of Pulsed Power Systems for Biomedical Applications,” 14<sup>th</sup> International Pulsed Power Conference, Dallas, TX, June 15-18, 2003.

X. Gu, P. Wijetunga, A. Kuthi, M. Behrend, P.T. Vernier and M. Gundersen, “Nanosecond Rise Time Minipulser for Cell Electroperturbation,” 14th International Pulsed Power Conference, Dallas TX June 15-18, 2003.

X. Gu, A. Kuthi, M. Behrend, P.T. Vernier, and M.A. Gundersen, “Compact Pulse Generator for Nanosecond Electroperturbation of Biological Cells,” 26<sup>th</sup> IEEE International Power Modulator Conference, San Francisco, CA, May 23-26, 2004.

Kuthi, P. Gabrielson, M. Behrend and M. Gundersen, “Nanosecond Pulse Generator Using a Fast Recovery Diode,” 26th IEEE Power Modulator Conference, San Francisco, CA, May 23-26, 2004.

Kuthi, M. Behrend, T. Vernier and M. Gundersen, “Bipolar Nanosecond Pulse Generation Using Transmission Lines for Cell Electromanipulation,” 26th IEEE Power Modulator Conference, San Francisco, CA, May 23-26, 2004.

P.T. Vernier, Y. Sun, L. Marcu, C.M. Craft, and M.A. Gundersen, “Nanosecond Pulsed Electric Fields Trigger Intracellular Signals in Human Lymphocytes,” Nanotech 2004, Boston, MA, March 7-11, 2004, Technical Proceedings of the 2004 NSTI Nanotechnology Conference and Trade Show, Vol. 1, Ch. 1, pp. 7-10, 2004.

Y. Sun, P.T. Vernier, M. Behrend, L. Marcu, and M.A. Gundersen, “Microscope Slide Electrode Chamber for Nanosecond, Megavolt-Per-Meter Biological Investigations”, Nanotech 2004, Boston, MA, March 7-11, 2004, Technical Proceedings of the 2004 NSTI Nanotechnology Conference and Trade Show, Vol. 1, Ch. 11, pp. 485-488.

P. T. Vernier, L. Marcu, Y. Sun, S. Salemi, C. M. Craft, and M. A. Gundersen, “Real-time imaging of mammalian cells in nanosecond, megawatt, millijoule pulsed electric fields”, BIOS 2004 (SPIE), San Jose, Jan. 2004.

#### Other Publications

C. Young, L. Marcu, M. Behrend, T. Vernier, M. Gundersen, A. Li, X. Zhu, C. Craft, “Introduction of Apoptosis in Mammalian Cells by Exposure to Electric Fields of Nanosecond Duration,” Proceedings of the 6th Annual Grodins Graduate Research Symposium, University of Southern California, Biomedical Engineering Department, March 23, 2002.

M. Behrend, “UPSET: Triggering natural cell death in cancer,” USC Illumin Magazine, 2003.

### **INTERACTIONS/TRANSITIONS**

#### **A. PARTICIPATION/PRESENTATIONS AT MEETINGS, CONFERENCES, SEMINARS, ETC.**

##### **Oral Presentations, Invited**

“Some Physics and Applications Involving Short, High Field Electrical Pulses,” presented by M. Gundersen at University of Southern California, Seminar in Condensed Matter Physics, June 21, 2002.

P. T. Vernier and M. A. Gundersen, “Ultrashort electric perturbations trigger membrane phospholipid translocation and apoptosis”, Air Force Research Laboratory, Wright-Patterson Air Force Base, Dayton, OH, 2002.

“Ultrashort-Pulsed Electroperturbation: Induced Caspase Activation in Human Lymphocytes,” presented by M. Gundersen, UC Irvine, CA, December 12, 2002.

M. Behrend and K. Chiu, “Ultrashort Pulse Systems Electroperturbation Technology,” USC W.V.T. Rusch Engineering Honors Program Student Research Presentations, 2002.

M. Gundersen, “Electric Field-Induced Apoptosis in Human Lymphocytes”, presented to Agilent management at USC, Feb. 6, 2003.

M. Behrend, “Ultra-short pulsed systems electroperturbation technology - UPSET,” presented at ARCS luncheon, Los Angeles, CA, March 28, 2003.

“Physics and Applications of Pulsed Power,” M. Gundersen, presented to the Physics Department of the Naval Postgraduate School, August 2003.

J. Lo, E.S. Kim, M.A. Gundersen, and L. Marcu, “MEMS Device for Fluorescence Spectroscopy Applications: Piezoelectrically Actuated Cantilever Grating Array,” 7th Annual Grodins’ Research Symposium, USC, May 2003.

M. Thu, K. Chiu, and A. Seegan, “Electrical Circuit Modeling of Biological Cells,” Undergraduate Research Symposium for Scholarly and Creative Work, University of Southern California, April 2003.

“Pulsed Power: Physics, and Two Diverse Applications”, M. Gundersen, Lawrence Berkeley Laboratory, February 17, 2004.

#### Contributed Oral Presentations, Conference

“Ultrashort-Pulsed Electroperturbation: Applications of High Pulsed Electric Fields to Induced Caspase Activation of Human Lymphocytes,” presented by M. Gundersen at AFOSR Bio-Inspired Concepts Review, Annapolis, MD, April 29-30, 2002.

“Ultrashort-Pulsed Electroperturbation: Applications of High-Pulsed Electric Fields to Induced Caspase Activation in Human Lymphocytes,” presented by M. Gundersen at High Voltage Workshop, Hollywood, CA, July 3, 2002.

Vernier, P. T., Zhu, X., Li, A., Marcu, L., Craft, C., Gundersen, M., “Ultrashort, High-field, Electric Pulses Trigger Membrane Phospholipid Translocation and Caspase Activation in Human Lymphocytes,” poster presented at Gordon Research Conference on Biochemistry, South Hadley, MA, July 21-26, 2002.

P. Wijetunga, X. Gu, A. Kuthi, P. T. Vernier, M. Behrend and M.A. Gundersen, “Electrical Modeling of Pulsed Power Systems for Biomedical Applications,” 14<sup>th</sup> International Pulsed Power Conference, Dallas, TX, June 15-18, 2003.

L. Marcu, Q. Fang, T. Papaioannou, J. A. Jo, P. Butte, B. Pikul, R. C. Thompson, W. H. Yong, K. L. Black, J. A. Freischlag, M. C. Fishbein, M. A. Gundersen, “Lifetime fluorescence spectroscopy for in-vivo diagnosis of tissues”, Keystone Symposia in Optical Imaging: Applications to Biology and Medicine, Taos, New Mexico Feb. 11-16, 2003.

Marcu L., P.T. Vernier, H.C. Manning, S. Salemi, A. Li, C.M. Craft, M. A. Gundersen, and D.J. Bornhop., “Fluorescence microscopy studies of a peripheral benzodiazepine receptor targeted molecular probe for brain”. Diagnostic Optical Spectroscopy, The European Conference on Biomedical Optics (Munich, Germany, June 2003).

P. T. Vernier, A. Li, L. Marcu, X. Zhu, C. M. Craft, and M. A. Gundersen, "Nanosecond, megawatt, millijoule pulses invert membrane phospholipids and activate caspases in malignant cells", ElectroMed 2003, San Antonio, 2003.

L. Marcu, P. T. Vernier, S. Salemi, M. Behrend, C. M. Craft, M. Gundersen, "Optical imaging of electroperturbative effects in Jurkat T lymphoblasts induced by ultrashort pulsed electric fields", World Congress in Medical Biophysics and Biomedical Engineering, Sydney, Australia 2003.

P. T. Vernier, A. Li, L. Marcu, X. Zhu, C. M. Craft, and M. A. Gundersen, "Nanosecond electroperturbation of malignant cells", World Congress on Medical Physics and Biomedical Engineering, Sydney, 2003.

“Non-invasive intracellular electroperturbation of human lymphocytes,” Vernier, P.T., Y. Sun, L. Marcu, S. Salemi, C. M. Craft, and M. A. Gundersen, “Workshop on High-Field Effects and Fast Pulse Responses in Bio-Systems, IEEE Conference on Electrical Insulation and Dielectric Phenomena, Albuquerque, 2003.

“Field-dependent nanosecond electroperturbation of Jurkat T lymphoblasts,” Vernier, P. T., Y. Sun, L. Marcu, C. M. Craft, and M. A. Gundersen, Scientific Conference, Society for Physical Regulation in Biology and Medicine, San Antonio, 2004.

“Nanosecond, megawatt, millijoule pulses selectively perturb but do not porate mammalian cells,” Vernier, P. T. and M. A. Gundersen, Air Force Office of Scientific Research, Chemistry and Life Sciences Directorate, Bio-Inspired Concepts Review, Annapolis, MD, 2003.

“Fluorescence microscopy studies of a peripheral benzodiazepine receptor-targeted molecular probe for brain tumor imaging,” Marcu, L., P. T. Vernier, C. H. Manning, S. Salemi, A. Li, C. M. Craft, M. A. Gundersen, and D. J. Bornhop, Diagnostic Optical Spectroscopy, European Conference on Biomedical Optics, Munich, Germany, 2003.

“Germination of *Bacillus atrophaeus* spores after exposure to ultra-short, high-field electric pulses,” Thu, M., P. T. Vernier, M. Behrend, S. Salemi, C. M. Craft, and M. A. Gundersen, ElectroMed 2003, San Antonio, 2003.

“Compact pulse generator for nanosecond electroperturbation of biological cells,” Gu, X., A. Kuthi, M. Behrend, P. T. Vernier, Q. Zhou, and M. A. Gundersen, IEEE 26<sup>th</sup> International Power Modulator Conference, San Francisco, 2004.

“A catheter electrode for ultra-short, high-field pulses,” Thu, M., M. R. Behrend, P. T. Vernier, Y. Sun, A. Kuthi, L. Marcu, C. M. Craft, and M. A. Gundersen, IEEE 26<sup>th</sup> International Power Modulator Conference, San Francisco, 2004.

“Real-time imaging of mammalian cells in nanosecond, megawatt, millijoule pulsed electric fields,” Vernier, P. T., L. Marcu, Y. Sun, S. Salemi, C. M. Craft, and M. A. Gundersen, BIOS 2004 (SPIE), San Jose, 2004.

“Nanosecond pulsed electric fields trigger intracellular signals in human lymphocytes,” Vernier, P. T., Y. Sun, L. Marcu, C. M. Craft, and M. A. Gundersen, Nanotech 2004, Boston, 2004.

“Nanoelectropulse perturbations of calcium and phospholipid distribution in human lymphocytes,” Vernier, P. T., Y. Sun, L. Marcu, C. M. Craft, and M. A. Gundersen, Bioelectromagnetics Society 26<sup>th</sup> Annual Meeting, Washington, 2004.

“CMOS-compatible MEMS on multi-project wafers: fabrication and characterization, National Institute of Standards and Technology,” Vernier, P. T., Semiconductor Electronics Division, Electronics and Electrical Engineering Laboratory, Gaithersburg, MD, 2003.

“Non-invasive approaches to nano-biology through advanced pulsed power,” Y. Sun, P. T. Vernier, M. Behrend, L. Marcu, and M. A. Gundersen, Workshop on High-Field Effects and Fast Pulse Responses in Bio-Systems, IEEE Conference on Electrical Insulation and Dielectric Phenomena, October 19-22, Albuquerque, 2003.

#### **B. CONSULTATIVE AND ADVISORY FUNCTIONS TO OTHER LABORATORIES AND AGENCIES**

**C. TRANSITIONS. DESCRIBE CASES WHERE KNOWLEDGE RESULTING FROM YOUR EFFORT IS USED, IN A TECHNOLOGY APPLICATION.**

#### **NEW DISCOVERIES, INVENTIONS, OR PATENT DISCLOSURES**

“Method For Intracellular Modifications Within Living Cells Using Pulsed Electric Fields

USC File No. 3237A

#### **HONORS/AWARDS**

Matthew Behrend began his graduate studies at USC in the Fall and is supported by three of the most prestigious national engineering awards:

Hertz Foundation Fellowship

National Defense Science and Engineering Graduate Fellowship.

Outstanding Electrical Engineering Student Award, given annually by Eta Kappa Nu, the national Electrical Engineering honor society.

In May he was awarded the Undergraduate Research Award at the International IEEE Power Modulator Conference. He also received this award in 2002.